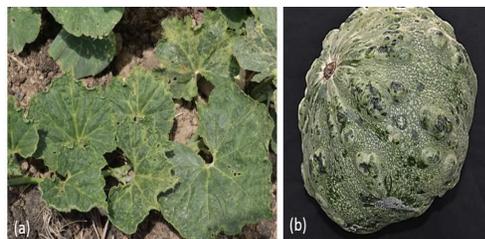


Diseases Caused by Viruses

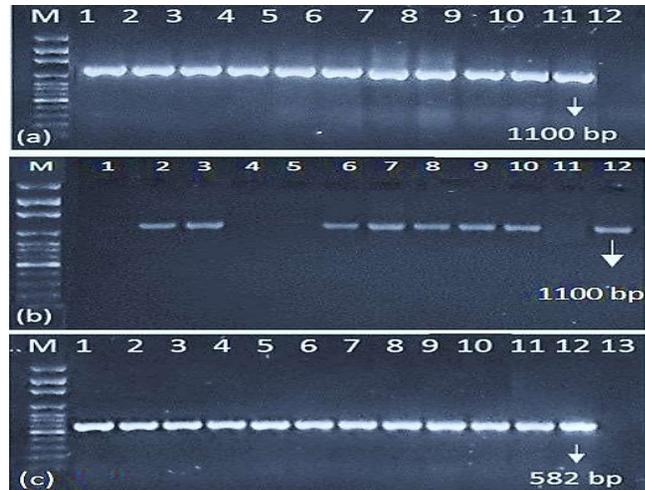
First Report of Zucchini yellow mosaic virus and Squash mosaic virus Infecting Cucurbits in Trinidad.C. Chinnaraja¹, A. Ramkissoon, R. Rajendran¹, S. T. Tony², A. Ramsubhag¹ and J. Jayaraj^{1*}¹Department of Life Sciences, Faculty of Science and Technology, The University of the West Indies, Trinidad. ²Ministry of Food Production, Trinidad and Tobago.Plant Dis. 100:1, 2016; published online as <http://dx.doi.org/10.1094/PDIS-09-15-0988>. PDN. Accepted for publication 9 November 2015.

Cucurbits are major food crops of the Caribbean region and cultivated in 27% of fields in Trinidad with an average production of ;2750 tons (pumpkin, squash, and gourds) per year (<http://faostat3.fao.org/browse/Q/QC/E>). For the past two years, we have noticed plants showing disease symptoms including severe mosaic and yellowing of leaves and severe deformation and color cracking in pumpkin and squash fruits in all the cultivars grown in Trinidad. The infection was suspected to be caused by Zucchini yellow mosaic virus (ZYMV) (Hasi'ow-Jaroszewska et al. 2013; Desbiez and Lecoq 1997). Detailed surveys to record the severity of disease were conducted during dry and rainy seasons for a two-year period in farmers' fields in six different locations (Barrackpore, Macoya, Las Lomas, Moruga, Waterloo, and Orange Groove) representing all the major cropping zones of the country. Virus infection was noticed at all growth stages of the crop and a maximum disease incidence of 74% was recorded in the dry season during 2014 and 2015. The leaf samples from 60 suspected pumpkin plants (10 per location) were collected from the fields, and total RNAs were isolated. RNA (1 µg) was used for cDNA synthesis and PCR was performed with ZYMV coat protein specific primers: CP-forward (59- GCTCCATACATAGCTGAGAC-39) and CP-reverse (59-AACGGAGTC TAATCTCGAGC-39) targeting a 1100-bp sequence. PCR was also performed to identify a possible coinfection with other viruses, such as Watermelon mosaic virus, Cucumber mosaic virus, Papaya ring spot virus, 240 additional samples of squash (60 plants), watermelon (60 plants), cucumber (60 plants), and bitter gourd (60 plants). Amplification of a ZYMV-specific band was found in 96% of pumpkin (58 of 60 samples) and in 60% of squash (36 of 60 samples). Interestingly, we found coinfection of Squash mosaic virus (SqMV) in 56% of pumpkin (34 of 60 samples) and in 50% of watermelon samples (30 of 60 samples) by using primers SqMV RNA2 f1 (GGTGCAGCAGCTTGGAAGCTTATAATCCAATTGG) and SqMV RNA2 r1 (TGGGAAAGAAGCCACAACAAACCC) targeting a 582-bp nucleotide sequence. In addition, high populations of aphids (*Aphis gossypii*) were noticed in cucurbit fields of Trinidad. To confirm the transmission of ZYMV through aphid vectors, 10 sterile pumpkin seedlings were grown in green house. Pumpkin seedlings naturally infected with ZYMV and confirmed by PCR reactions were collected from a Barrackpore field and grown in greenhouse. Single adult aphids (*Aphis gossypii*) from a virus-free colony were transferred to these infected seedlings for 48 h for acquisition feeding and then transferred to the 10 sterile pumpkin seedlings for 48 h for inoculation feeding in a netted greenhouse. After seven days, total RNAs were extracted from leaf samples of all the seedlings and it is followed by PCR using ZYMV-specific primers (CR-for/CP-rev). The 1,100-bp band was amplified from eight out of 10 receptor seedlings, which confirmed the presence and transmission of ZYMV in pumpkin. All the PCR

products of ZYMV and SqMV were purified, sequenced directly, and submitted to GenBank as Accession Nos. KT224395 to KT224404 (ZYMV) and KT291731 to KT291734 (SqMV). BLAST search of these sequences revealed 98% sequence identity with ZYMV isolate C-16 (DQ645729) from southeastern Spain and 95% identity with isolate SqMV-Z (AF059532) from Arizona. This report indicates the occurrence of ZYMV in pumpkin and squash plants and also SqMV in pumpkin and watermelon plants in Trinidad.



Supplementary Figure 1. Pumpkin plant on farmer field showing severe symptoms of ZYMV on their leaves with severe mosaic and yellowing (a) and fruits with deformation at Barrackpore, Trinidad.



Supplementary figure 2. Amplification of ZYMV from pumpkin (a) and squash (b) samples from Trinidad. Lane M;100 bp ladder, Lane 1-2; samples from Barrackpore, 3-4; Macoya, 5-6; Las lomas, 7-8; Moruga, 9-10; Orange groove. 11(a), 12(b); positive control, 12(a),11(b); negative control, (c); Amplification of SqMV from pumpkin (1-6) and watermelon (7-10) samples. Lane 1-3, 7-8; Barrackpore, 4-6, 9-10; Mayaro.

Acknowledgments

Financial assistance received from ACP-EU is gratefully acknowledged.

References:

- Hasi'ow-Jaroszewska, B. 2013. Acta Sci. Pol. Hortorum Cultus 12:75.
Desbiez, C., and Lecoq, H. 1997. Plant Pathol. 46:809.